Genomic analysis of high-risk prostate cancer

Key words: high-risk prostate cancer, exome, transcriptome, next-generation sequencing, hydroxymethylation

Summary

Whole exome sequencing was performed on 38 high-risk prostate cancer (PCa) samples. We confirmed recurrent mutations in PCa-specific genes, but also identified genes not reported to be mutated, like TET1. This DNA hydroxymethylase converts methylcytosines to hydroxymethylcytosines as a first step in DNA demethylation. By immunohistochemistry, we detected decreased TET1 protein levels in tumor compared to surrounding non-tumor tissue. DNA hydroxymethylation followed the same course. Furthermore, TET1 mRNA expression levels are an independent predictor of metastasis-free survival in a larger retrospective cohort, indicating an important role for TET1 and hydroxymethylation in PCa.

The LNCaP and C4-2B cell lines form an excellent preclinical model to study the development of metastatic castration-resistant PCa. Both exome and transcriptome sequencing was performed: more than half of the mutations found in the exomes were confirmed in the RNA-seq data. Combining C4-2B-specific mutations with differentially expressed genes allowed the detection of changes in focal adhesion and ECM-receptor interactions, which might contribute to the metastatic potential of C4-2B cells.

Introduction

Prostate cancer (PCa) is the second most frequently diagnosed cancer in males worldwide.[1] A range of genomic alterations, including point mutations, copy number changes and rearrangements, can lead to cancer development.[2] Due to the heterogeneity of PCa, it still remains a clinical challenge to differentiate indolent from aggressive tumors. A better molecular profiling of the tumors should enable improved disease classification, ultimately providing information that could direct a more personalized treatment. One approach is to study the contribution of somatic point mutations to the oncogenic process, which we did in high-risk primary tumors and cell line models.

Molecular classification of prostate cancer

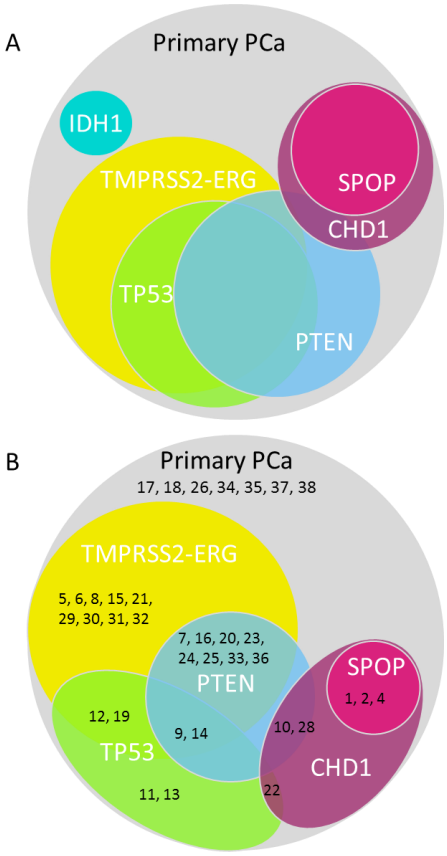
DNA was extracted from the primary tumors and their matched normal of 47 patients with high-risk PCa (HRPC). Tumors with PSA > 20 ng/ml, or Gleason score ≥ 8 or clinical stage ≥ T2c are known to have a high risk on disease recurrence after treatment.[3] We characterized 38 samples by copy number profiling and exome sequencing, the latter detecting an average of 22 mutations per patient.

Large scale integration of copy number aberrations and mutations has led to the definition of seven molecular subclasses of PCa.[4, 5] This classification might start the transition from a clinically heterogeneous disease to a collection of homogeneous subtypes identified by molecular features (Figure 1A). Each subclass might have a distinct prognosis, and distinct targeted therapies. The classification, based on over 300 samples, is defined by the following features:

- 3 subtypes are characterized by gene fusions involving different members of the ERG gene family.

- Inactivating point mutations or copy number deletions of PTEN (12 of 38 in our cohort).
- SPOP mutations, frequently co-occurring with deletion of the CHD1 gene (3 of 38 in our cohort).
- IDH1 mutations or loss of expression (no mutations found in our cohort).
- A subclass covering all other (26%) (7 of 38 in our cohort).

Our cohort of 38 samples for which both exome sequencing and copy number profiling was available is in concordance with this classification (Figure 1B).



Genomic analysis of HRPC reveals loss-of-function mutation in RFC1

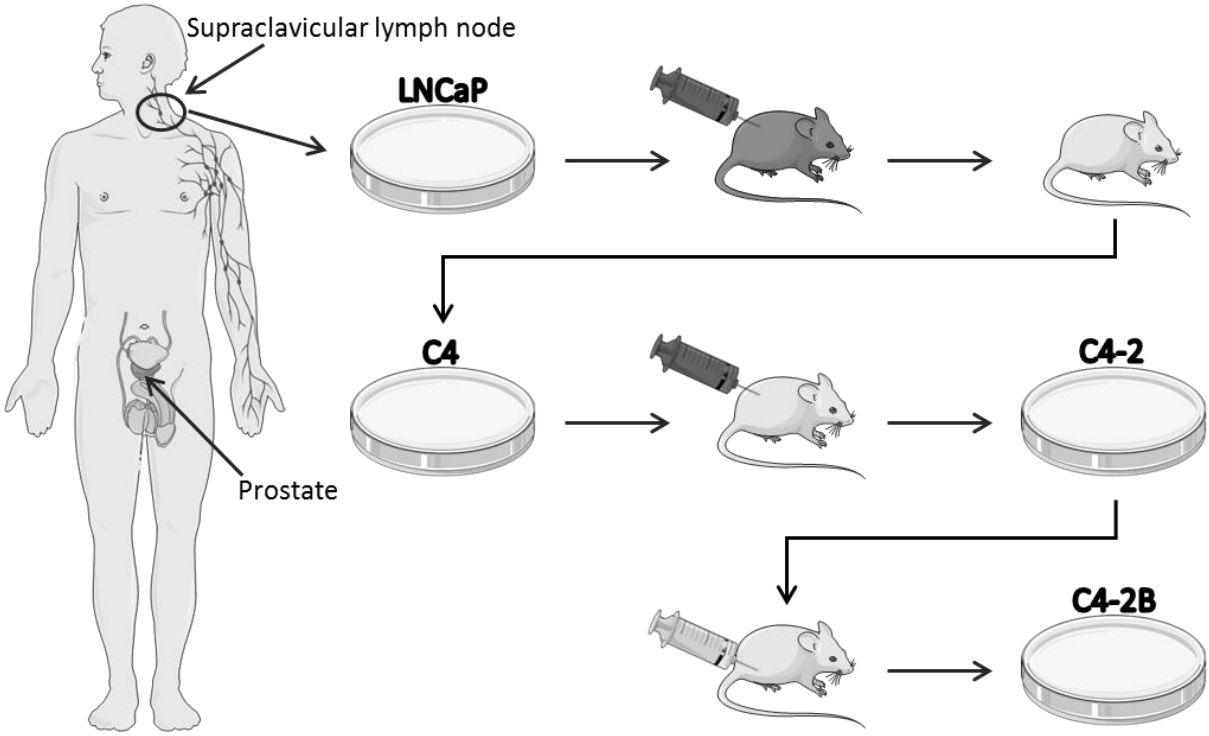
One HRPC sample revealed 451 mutations in its exome, making it an outlier in our study. This could indicate a compromised DNA repair. Indeed, one of the mutations was located in the DNA-repair gene *Replication Factor C*. *In silico* calculations predict that this mutation affects the interaction of RFC1 with PCNA and hence the recruitment of DNA polymerase to sites of damaged DNA.

Hydroxymethylation and TET1 are affected in HRPC

Another sample of our cohort contained a novel point mutation in the *TET1* gene. Since copy number profiling revealed that the region containing this gene was lost in six additional samples, we specifically focused on this gene.[6] The *TET1* (Ten-Eleven Translocation 1) gene encodes a dioxygenase that converts 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), which is the first step in cytosine demethylation, and is thus linked with changes in gene expression or chromatin organization.[7] It is well known that DNA methylation is profoundly altered in cancer, but the mechanisms behind methylation dynamics are not yet fully understood. We would propose a role for TET1 here.

Immunohistochemistry for TET1 and 5hmC showed reduced levels in tumor versus non-tumor prostate tissue in 39 and 27 of 40 cases respectively. A retrospective cohort of 235 HRPC samples was used to study the clinical relevance of TET1 levels. This revealed that TET1 mRNA expression is an independent predictor of metastases-free survival. With a hazard ratio of 2.12, patients with low TET1 have a doubled risk at developing metastases, proving that TET1 is as powerful as high Gleason score in predicting bad prognosis. Moreover, we performed the first exploratory (h)MeDIP-Seq analyses of eight HRPC samples. For these analyses, methylated and hydroxymethylated DNA was immunoprecipitated and deep-sequenced. We detected a large heterogeneity in hydroxymethylation changes in tumor versus non-tumor genomes and changes in 5hmC were detected in genes linked with cell polarity determination.

Comparative genomics and transcriptomics of LNCaP and C4-2B cell lines



The progression of PCa from androgen-dependent to androgen-independent poses an important clinical question, as the mechanisms leading to metastatic PCa are not well understood. C4-2B cells were derived from a bone metastasis that grew in nude mice after inoculation with LNCaP-derived, castration-resistant C4-2 cells (Figure 2).[8, 9] LNCaP and C4-2B cells thus form an excellent model to study the development of metastatic castration-resistant PCa. Because of the importance of this progression model, we characterized both cell lines using exome and transcriptome sequencing. Exome sequencing detected 2188 and 3840 mutations in LNCaP and C4-2B cells respectively, of which 1784 were found in both cell lines.[10, 11] More than half of the mutations found in the exomes of both cell lines were confirmed with transcriptome sequencing. The transcriptome data also revealed 457 and 246 genes with increased and decreased expression respectively in C4-2B compared to LNCaP cells. Based on the C4-2B-specific point mutations and the differentially expressed genes, we detected changes in the focal adhesion and ECM-receptor interaction pathways. Whether these contribute to the metastatic potential of C4-2B cells remains to be investigated.

Conclusions

Exome sequencing of our HRPC cohort confirms the seven molecular subtypes. In addition, the discovery of a *TET1* mutation led to the description of a subclass of HRPC with changes in the hydroxymethylation pathway. The diagnostic, prognostic or even therapeutic possibilities now need to be investigated.

Key messages for daily practice

1. HRPC has recurrent mutations in PTEN, p53, CHD1, SPOP, FoxA1 and ERG.

2. HRPC has lower levels of global hydroxymethylation; the implications for cancer biology will be studied in preclinical models first.
3. Low TET1 expression levels are correlated with worse metastases-free survival, but clinical relevance needs to be further validated.

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Figure Legends

Figure 1. Molecular classification of high-risk PCa. **A.** About half of all PCas harbor TMPRSS2-ERG fusions. PTEN and TP53 are deleted or mutated in 20-40% of primary PCa, with significant overlap with each other and with the fusion. SPOP mutations occur in about 10% of PCas and are mutually exclusive with the fusion, while they are associated with CHD1 deletions. Picture adapted from Barbieri *et al.* **B.** Same classification as in A, but applied to our 38 high-risk PCa samples. TMPRSS2-ERG means that ERG overexpression was detected using immunohistochemistry.

Figure 2. The development of LNCaP and C4-2B PCa cell lines. LNCaP cells were isolated from a supraclavicular lymph node metastasis. The C4-2B cell line is derived from a LNCaP tumor grown in castrated mice. Intact and castrated mice are represented in dark and light gray respectively. A dark syringe represents subcutaneous injection of PCa cells with human fibroblasts. A light syringe indicates orthotopic injection of PCa cells only.

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Disclosure statement

The authors have nothing to disclose and indicate no potential conflict of interest.